polynucleotides are substantially complementary to each other and each comprising a homology clamp that substantially corresponds to or is substantially complementary to at least a portion of said preselected DNA sequence of said nucleus; and

(b) transplanting said nucleus into an oocyte to produce a recombinant zygote.

Please cancel Claims 38 and 40 without prejudice to Applicants' right to pursue the subject matter of these claims in one or more continuation, continuation-in-part or divisional applications.

REMARKS

Claims 1-38 and 40 are pending in the instant application. Claim 1-38 and 40 stand rejected under 35 U.S.C. § 112, first paragraph as lacking an enabling disclosure; Claims 1-21 stand rejected under 35 U.S.C. § 103(a) as being obvious over Campbell *et al.* (1996) *Nature* 380, 64-66 ("Campbell *et al.*") or Cibelli *et al. Science* 280, 1256-1258 ("Cibelli *et al.*") in view of U.S. Pat. No. 5,763,240 to Zarling *et al.* ("Zarling *et al.*"); and Claims 22-37 stand rejected under 35 U.S.C. § 103(a) as being obvious over Kimura *et al.* (1995) *Biology of Reprod.* 52, 709-720 ("Kimura *et al.*") in view of Zarling *et al.*

Claim 1 has been amended to recite with more particularity that which Applicants regard as their invention. The amendment is not intended to and is believed not to change the scope of the claim. Claims 38 and 40 have been cancelled without prejudice to Applicants' right to pursue the subjection matter of these claims in one or more continuation, continuation-in-part or divisional applications. A "Marked-Up Version" is attached hereto to show the amendments to the present claims.

Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 1-38 and 40 stand rejected under 35 U.S.C. § 112, first paragraph as lacking an enabling disclosure. Claims 38 and 40 have been cancelled by the instant amendment, thereby obviating the rejection of these claims.

Claim 1 recites altering a preselected DNA sequence of a donor nucleus by introducing a pair of substantially complementary single-stranded targeting polynucleotides, and a recombinase into the donor nucleus and transplanting the altered nucleus into an oocyte to produce a recombinant zygote. The pair of targeting polynucleotides each comprise a homology clamp that substantially corresponds to or is substantially complementary to at least a portion of the preselected DNA sequence of the nucleus.

Claim 22 recites introducing a spermatozoa, a pair of substantially complementary single-stranded targeting polynucleotides each having a homology clamp that substantially corresponds to or is substantially complementary to a predetermined DNA sequence of the spermatozoa and/or said oocyte, and a recombinase into an oocyte, whereby a recombinant zygote is produced.

The Examiner stipulates that the specification enables altering a preselected DNA sequence in a nuclear chromosome by introducing into a donor nucleus a recombinase, and a pair of substantially complementary targeting polynucleotides, each having a homology clamp substantially corresponding to or complementary to at least a portion of the preselected DNA sequence. Paper No. 8 at page 2, ¶ 2.

However, the Examiner argues that no evidence of record indicates that an altered chromosomal sequence would appear as a phenotypic change in an altered zygote or offspring. *Id.* The Examiner argues further that if the animal does not have the desired phenotype, then the claimed method has no patentable use. Thus, the Examiner concludes, the skilled artisan would have required an undue amount of experimentation without a predictable degree of success to practice the claimed invention. Applicants respectfully disagree.

The specification and prior art clearly enable targeting and altering a pre-selected nuclear DNA sequence by introducing targeting polynucleotides and a recombinase, as claimed in the present invention. This much is clear from the Examiner's stipulation and the issued claims in U.S. Pat No. 5,763,240. The cited prior art, Campbell *et al.* and Cibelli *et al.*, enable cloning of live mammalian offspring by transferring donor nuclei from a cell line

to enucleated oocytes. Additionally, Cibelli *et al.* enable producing a transgenic animal by transferring a genetically modified nucleus to an enucleated mature oocyte. Moreover, co-pending U.S. Serial No. 09/079,877 (published as WO 99/60108) enables producing a transgenic animal by introducing a recombinase and two substantially complementary targeting polynucleotides into embryos, thereby resulting in a transgenic animal with a targeted alteration in a preselected DNA sequence. *See* U.S. Ser. No. 09/079,877 (pg:ln) 94:20-99:9 (which pages are attached for the Examiner's convenience).

The Examiner suggests that the skilled artisan, using the claimed methods, would have no reasonable expectation that the recombinant zygote or animal would have a phenotypic change, and therefore, the artisan must use undue experimentation to achieve and/or find the phenotypic change. In the first instance, the present invention results in a much higher recombination rate than the random recombination achieved by the prior art. For example, Zarling *et al.* achieved an 11% increased recombination rate by introducing targeting polynucleotides and a recombinase as compared to 0% recombination by introducing only targeting polynucleotides. U.S. Pat. No. (col:ln) 22:48-63. Thus, the present invention results in a much higher recombination rate, a much higher rate of producing a recombinant zygote or animal having the targeted alteration. Consequently, the present invention results in a much lower amount of experimentation to achieve a recombinant zygote or animal with a desired alteration as compared to the prior art methods. Additionally, it is noted that the claims are not limited to obtaining a phenotypically modified zygote or animal. Rather, the claims cover *methods* for producing a recombinant zygote or animal, which means a zygote or animal possessing the targeted DNA sequence modification. The skilled artisan will readily appreciate that methods to produce a recombinant zygote or animal with a targeted modification at a much higher rate than previously provided will find immense use both at the genetic and protein levels.

Furthermore, as shown in co-pending U.S. Ser. No.09/079,877, pages 94-99, the methods of enhanced homologous recombination have resulted in altered phenotypes. For example, transgenic mice with altered phenotypes have been made.

Thus, all of the steps of Claims 1-37 are fully enabled by the specification and the prior art. The skilled artisan knows how to make a targeted modification to a preselected nuclear DNA sequence by introducing a pair of substantially complementary targeting polynucleotides and a recombinase to a donor nucleus. The skilled artisan knows how to transplant the nucleus into an oocyte to obtain a recombinant zygote.

Rejection Under 35 U.S.C. § 103(a)

Claims 1-21

Claims 1-21 stand rejected under 35 U.S.C. § 103(a) as being obvious over Campbell *et al.* or Cibelli *et al.* in view of Zarling *et al.*

Claim 1 recites altering a preselected DNA sequence of a donor nucleus by introducing a pair of substantially complementary single-stranded targeting polynucleotides, and a recombinase into the donor nucleus and transplanting the altered nucleus into an oocyte to produce a recombinant zygote. The pair of targeting polynucleotides each comprise a homology clamp that substantially corresponds to or is substantially complementary to at least a portion of the preselected DNA sequence of the nucleus.

The Examiner notes that Campbell *et al.* and Cibelli *et al.* teach the production of a cloned animal by transferring donor nuclei from a cell line to enucleated oocytes. The Examiner also notes that Zarling *et al.* teach enhanced targeted altering of a pre-selected DNA sequence by introducing to a nucleus a recombinase and two substantially complementary targeting polynucleotides each having a mutation of interest as compared to the targeted sequence and a homology clamp substantially complementary to the targeted sequence.

The Examiner argues it would have been obvious to the skilled artisan to use the methods of Zarling *et al.* to generate a donor nucleus with an alteration in a pre-selected DNA sequence, and use this altered nucleus as the donor nucleus in the nuclear transfer techniques taught by Campbell *et al.* and Cibelli *et al.*. Thus, the Examiner concludes the claimed invention is obvious over Campbell *et al.* or Cibelli *et al.* in view of Zarling *et al.* The

Applicants respectfully disagree.

When rejecting claims under 35 U.S.C. § 103, the Examiner bears the burden of establishing a *prima facie* case of obviousness. *See, e.g., In re Bell*, 26 USPQ2d 1529 (Fed. Cir. 1993); M.P.E.P. § 2142. To establish a *prima facie* case, three basic criteria must be met, one of which is that the prior art must provide one of ordinary skill with a suggestion or motivation to modify or combine the teachings of the references relied upon by the Examiner to arrive at the claimed invention.

The Examiner has failed to establish a *prima facie* obviousness against Claim 1 because, at a minimum, there is no motivation within the references or the general knowledge of the skilled artisan to combine the references in the manner suggested by the Examiner. Applicants agree that Campbell *et al.* and Cibelli *et al.* teach nuclear transfer techniques to generate cloned animals. However, nowhere in these two references does it even mention the desirability to use an enhanced homologous recombination technique to provide a donor nucleus with an altered predetermined nuclear DNA sequence. Applicants also agree that Zarling *et al.* teach a method of producing a cell with an altered predetermined nuclear DNA sequence by introducing to the nucleus a recombinase and two substantially complementary polynucleotides each having a mutation of interest as compared to the targeted sequence and a homology clamp substantially complementary to the targeted sequence. However, nowhere does Zarling *et al.* discuss the desirability of producing recombinant zygotes or animals by using the altered nucleus as a donor nucleus. Only the present invention teaches that such is desirable. However, the Federal Circuit has repeatedly warned that the requisite motivation to combine or modify references must come from the prior art, not the applicant's specification. *See, e.g., In re Dow Chem. Co.*, 5 USPQ2d 1529, 1531-32 (Fed. Cir. 1988). The mere fact that references can be modified does not render the resultant modification obvious unless the prior art also provides the motivation to combine or modify the references to arrive at the claimed invention. *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990); MPEP § 2143.01.

Thus, for this reason alone the Examiner has failed to establish a *prima facie* case of

obviousness against Claim 1. Claims 2-21 ultimately depend from Claim 1 and contain each and every limitation thereof. It is axiomatic patent law that a claim depending from a patentable claim is itself patentable over the prior art. Thus, the Examiner has also failed to establish a *prima facie* case of obviousness against Claims 2-21 for the same reasons she failed to do so for Claim 1.

Accordingly, Applicants respectfully request the rejection of Claims 1-21 under 35 U.S.C. § 103(a) be withdrawn.

Claims 22-37

Claims 22-37 stand rejected under 35 U.S.C. § 103(a) as being obvious over Kimura *et al.* in view of Zarling *et al.*

Claim 22 recites introducing a spermatozoa, a pair of substantially complementary single-stranded targeting polynucleotides each having a homology clamp that substantially corresponds to or is substantially complementary to a predetermined DNA sequence of the spermatozoa and/or said oocyte, and a recombinase into an oocyte, whereby a recombinant zygote is produced.

The Examiner notes that Kimura *et al* teach producing mouse zygotes by introducing a mouse sperm into an oocyte by piezo-driven micropipette. The Examiner stipulates that Kimura *et al.* do not teach or suggest producing a recombinant mouse by introducing into the oocyte a sperm, a recombinase and two substantially complementary targeting polynucleotides each having a mutation of interest as compared to the targeted sequence and a homology clamp substantially complementary to the targeted sequence. The Examiner also notes that Zarling *et al.* teach enhanced targeted altering of a pre-selected DNA sequence by introducing to a nucleus a recombinase and two substantially complementary targeting polynucleotides each having a mutation of interest as compared to the targeted sequence and a homology clamp substantially complementary to the targeted sequence. The Examiner argues it would have been obvious to the skilled artisan to use the methods of Zarling *et al.* in combination with that of Kimura *et al.* to introduce into the oocyte a sperm, a recombinase

and two substantially complementary targeting polynucleotides each having a mutation of interest as compared to the targeted sequence and a homology clamp substantially complementary to the targeted sequence. Applicants respectfully disagree.

The Examiner has failed to establish a *prima facie* obviousness against Claim 1 because, at a minimum, there is no motivation within the references or the general knowledge of the skilled artisan to combine the references in the manner suggested by the Examiner. Applicants agree that Kimura *et al.* teach producing mouse zygotes by piezo-driven micropipette introduction of a mouse sperm into a mouse oocyte. However, nowhere does this reference even mention the desirability to use an enhanced homologous recombination technique to alter a predetermined nuclear DNA sequence within the oocyte and/or the sperm. Applicants also agree that Zarling *et al.* teach a method of producing a cell with an altered predetermined nuclear DNA sequence by introducing to the nucleus a recombinase and two substantially complementary polynucleotides each having a mutation of interest as compared to the targeted sequence and a homology clamp substantially complementary to the targeted sequence. However, nowhere does Zarling *et al.* discuss the desirability of producing recombinant zygotes or animals by introducing the recombinase and two substantially complementary targeting polynucleotides with the sperm into an oocyte. Only the present invention teaches that such is desirable. However, the Federal Circuit has repeatedly warned that the requisite motivation to combine or modify references must come from the prior art, not the applicant's specification. *See, e.g., In re Dow Chem. Co.*, 5 USPQ2d 1529, 1531-32 (Fed. Cir. 1988). The mere fact that references can be modified does not render the resultant modification obvious unless the prior art also provides the motivation to combine or modify the references to arrive at the claimed invention. *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990); MPEP § 2143.01.

Thus, for this reason alone the Examiner has failed to establish a *prima facie* case of obviousness against Claim 22. Claims 22-37 ultimately depend from Claim 22 and contain each and every limitation thereof. It is axiomatic patent law that a claim depending from a patentable claim is itself patentable over the prior art. Thus, the Examiner has also failed to

establish a *prima facie* case of obviousness against Claims 23-37 for the same reasons she failed to do so for Claim 22.

Accordingly, Applicants respectfully request the rejection of Claims 22-37 under 35 U.S.C. § 103(a) be withdrawn.

CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and an early notification of such is solicited. If, upon review, the Examiner feels there are additional outstanding issues, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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MARKED-UP VERSION

1. (Amended) A method comprising:

(a) altering a [chromosomal] preselected DNA sequence of a donor nucleus of a donor cell by introducing a pair of single-stranded targeting polynucleotides, and a recombinase into said donor nucleus of said donor cell, wherein said pair of targeting polynucleotides are substantially complementary to each other and each comprising a homology clamp that substantially corresponds to or is substantially complementary to [a predetermined] at least a portion of said preselected DNA sequence of said nucleus; and[,]

(b) transplanting said nucleus into an oocyte to produce a recombinant zygote.

upon digestion with EcoR1 cssDNA:probe target hybrids can be completely cut, as shown in Figure 18 A and C, Lane 2. When similar reactions are performed with uncut pRD.59 targets, we found that not all of the probe:target hybrids are relaxed as with pRD.0 targets, as judged by the appearance of two bands corresponding to a pRD59 I* hybrid, where the hybrids co-migrate with Form I supercoiled DNA and a pRD59 rI* hybrid that migrates with relaxed targets (Figure 18B and D, Lane 3). When these hybrids are digested with EcoRI we find that the pRD59 rI* hybrid is more susceptible to EcoRI cleavage than the pRD59 rI* hybrid (Figure 18B and D, Lane 4). This shows that there is a restoration of the EcoRI site in relaxed targets, but not in the non-relaxed I* hybrid. Since pRD59 targets do not contain an EcoRI site, cleavage by EcoRI can only be explained by re-annealing of cssDNA probe IP290 within the IP290 probe:target pRD59 hybrid.

To further characterize the structural differences between pRD59 rI* hybrids and pRD59 I* hybrids, cssDNA probe:target hybrids were formed between IP290 and pRD59, deproteinized and thermally melted for 5 mins at 37°C, 45°C, 55°C, and 65°C, respectively. Figure 19 shows that pRD59 rI* hybrids are more thermostable than pRD59 I* hybrids. For both types of hybrids probe:target hybrids are completely dissociated after heating to 95°C (data not shown). Taken together these data support the structures of our models for hybrids (Figure 13).

EXAMPLE 6

Homologous recombination targeting in fertilized mouse zygotes

Ornithine transcarbamylase (OTC) is a mitochondrial matrix enzyme that catalyzes the synthesis of citrulline from ornithine and carbamylphosphate in the second step of the mammalian urea cycle. OTC deficiency in humans is the most common and severe defect of the urea cycle disorders. OTC is an X-linked gene that is primarily expressed in the liver and to a lesser extent in the small intestine. Affected males develop hyperammonemia, acidosis, orotic aciduria, coma and death occurs in up to 75% of affected males, regardless of intervention. Two allelic mutations at the OTC locus are known in mice: spf and spf-ash, (sparse fur--abnormal skin and hair). In addition to hyperammonemia and orotic aciduria, spf-ash mice can be readily identified by the

abnormal skin and hair phenotype. The spf-ash mutation is a single-base substitution at the end of exon 4 that results in alternative intron-exon splicing to produce an aberrant non-functional elongated pre-mRNA. Because of the clinical importance of OTC defects in humans, there is an intensive effort to develop in vivo methods to correct the enzymatic defect in the spf-ash mouse model.

We used the murine spf-ash model of OTC deficiency to test the ability of RecA-coated complementary single-stranded DNA (css) OTC probes to target and correct a single-base substitution mutation in fertilized mouse zygotes. A 230 bp RecA-coated cssDNA probe amplified from the normal mouse OTC gene was microinjected into embryos derived from matins of B6C3H homozygous spf-ash female with normal B6D2F1J males. After re-implantation of 75 embryos that were microinjected with RecA-coated cssDNA into CD1 foster mothers, 25 developmentally normal pups (17 female and 8 male) were born. Sequence analysis of the genomic DNA isolated from tails of the male pups show that 3 out of 8 males were mosaic for a homologous recombination event at the spf-ash site in exon4 of the mouse OTC gene. Subsequent breeding of the three founder males with normal females resulted in normal female F_1 progeny, thus demonstrataing germline transmission of the homologous recombinant allele as well as phenotypic correction in F_1 animals. These homologoous recombinant changes were stable in F_2 and subsequent generations. These studies illustrate cssDNA mediated high frequency homologous recombination in fertilized mouse zygotes to create subtle genetic modifications at a desired target site in the chromosome.

Preparation of RecA-coated probe: A 230 bp fragment from the normal mouse OTC gene was amplified by PCR with primers M9 and M8 from pTAOTC (Figure 20). The PCR fragment was purified on Microcon-100 columns (Amicon) and then extensively dialyzed. The M9-M8 amplicon was denatured by heating the fragments to 98°C and then coated with RecA protein (Boehringer-Mannheim) at a ratio 3 nucleotides/ protein monomer. The final concentration of RecA-coated DNA in coating buffer (5 mM TrisOAc, pH 7.5, 0.5 mM DTT, 10 mM MgOAc, 1.22 mM ATPγS, 5.5 μM RecA) was 5 ng/ μL. RecA-coated filaments were made on the day of microinjection and then stored on ice until use.

Transgenic Mice: Five superovulated B6C3H (spf-ash/spf-ash) 5-7 week old females (Jackson Labs) were mated with five B6D2F1 males (Jackson Labs). Approximately 80-100 embryos were isolated from oviducts as described in Hogan et al. (1988). The female pronucleus of fertilized embryos was microinjected with 1-2 pl of RecA-coated M9-M8 cssDNA probe (5 ng/μL). Approximately 75 embryos survived the microinjection procedure and were then re-implanted into a total of three CD1 pseudopregnant foster mothers (Charles River). Pseudopregnant females were produced by mating foster mothers with vasectomized CD1 males (Charles River).

DNA Analysis: Tail biopsies were taken from all founder mice after weaning at three weeks of age. Genomic DNA was isolated from tail biopsies using standard procedures. To obtain the sequence of the DNA at the OTC locus, genomic DNA was amplified with PCR using primers M10-M11 or M54-M11 that flank the cssDNA probe sequence to generate a 250 bp or 314 bp amplicon (Figure 20). PCR fragments were sequenced manually using the Cyclist Exo Kit (Stratagene), automatically on an Applied Biosystems Model 373A sequencer, or by a MALDI-TOF mass spectrometry system (GeneTrace Systems, Menlo Park, CA)

Fertilized zygotes microinjected with RecA-coated DNA are viable. Plasmid pTAOTC1 carries a 250 bp segment of exon4 and surrounding intron sequences from the normal mouse OTC gene. A 230 bp cssDNA probe OTC1 was prepared by PCR amplification of pTAOTC1 with primers M9 and M8. cssDNA probe OTC1 was denatured and coated with RecA protein as described herein.

Homozygous spf-ash/spf-ash female and hemizygous (spf-ash/y) males can be phenotypically identified by the appearance of sparse fur and wrinkled skin early in development. A cross between homozygous spf-ash/spf-ash B6C3H females and normal B6D2F1 males yields heterozygous phenotypically normal females and hemizygous males with sparse fur and wrinkled skin. The RecA-coated cssDNA OTC probe was microinjected into embryos made from the cross of B6C3H homozygous female spf-ash and normal males. The female pronucleus of approximately 80-90 fertilized zygotes was microinjected with 2 pl of a 5ng/μL solution of RecA-coated cssDNA probe OTC1. Of

these, 75 embryos survived the microinjection procedure. To demonstrate that embryos that have been microinjected with RecA-coated cssDNA are viable, the embryos were re-implanted into three pseudopregnant CD1 foster mothers. From this, 25 developmentally normal pups (17 female and 8 male) were produced. All of the female mice were phenotypically normal. The eight male mice (mouse # 7, 14,16,17,22,23,24, and 25) were all affected with sparse-fur and wrinkled skin to various degrees.

RecA-coated cssDNA probe OTC1 recombines with the homologous chromosomal copy of the OTC gene in fertilized mouse zygotes. To determine the genotypes of the 25 founder mice produced from microinjected embryos, genomic DNA was isolated from tail biopsies. Genomic DNA was amplified with either the primer set M10-M11 or M54-M11 to produce either a 250 bp or 314 bp amplicon. By using these primer sets that flank the OTC1 probe, the DNA amplicon represents DNA from the endogenous OTC gene. PCR fragments from all of the eight mice and several female mice were sequenced to determine the base sequence at the spf-ash locus to determine if a normal allele (G) or a mutant allele (A) was present in the genomic DNA. Figure 21 shows sequencing gels of representative reactions. The panel on the left side shows the sequence of the homozygous spf-ash females that donated the eggs to produce the fertilized zygotes where only the mutant base A is present at the spf-ash locus, as expected. The sequence of female mouse #8 that should be heterozygous shows the presence of equal amounts of the bases G and A as expected. Male mice 7, 14 (shown), 23, 24,and 25 all showed only the mutant base A at the spf-ash locus, however male mice 16, 17, and 22 (shown) displayed both G (normal) and A (mutant) at the spf-ash locus.

To eliminate the possibility of PCR artifacts during PCR cycle sequencing the base compositions of the samples was independently confirmed by mass spectrometry sequencing (GeneTrace, Menlo Park). The relative (%) amounts of the A:G base composition at the spf-ash locus was also quantified and determined to be 70%:30% for samples from mouse #16 and #17 and 10%:90% for mouse #22. Since OTC is an X-linked gene the presence of mixed bases in male mice is likely the result of the mosaic animals produced of a mixture of mutant and gene corrected embryonic cells.

Germline transmission of the gene corrected OTC allele. To determine if the gene corrected allele in the mosaic male founder mice (#'s 16, 17, and 22) could be passed through to the germline, these mice and a control hemizygous mutant male (#7) were bred with normal B6D2F1 females. In this cross, if the male donates a mutant spf-ash X chromosome, then the resulting female progeny will be heterozygous spf-ash mutants. However if the male donates a normal (gene corrected) X chromosome the female progeny will be homozygous normal. In both cases the resulting F1 females will be phenotypically normal. The results of these crosses are summarized in Figure 22. In the control cross of hemizygous mutant male #7 with B6D2F1 females, all 14 female progeny were heterozygous, as expected. In test crosses of mosaic male mouse #17 and #22 with normal females, all resulting female progeny (5 and 9, respectively) were heterozygous. However in the cross with mosaic male mouse #16, one out nine total female progeny was a homozygous normal female (mouse # 213), as determined mass spectrometry DNA sequencing (GeneTrace, Menlo Park), demonstrating the gene corrected allele in founder mouse #16 was tranmitted through the germline.

To further verify that the F1 mouse #213 was, in fact, a germline-transmitted gene corrected homozygous normal female, this mouse and a control heterozygous spf-ash/+ mouse were bred with normal males. In the control cross B with the heterozygous female, 50% of the resulting male F2 progeny should be mutant spf-ash/y hemizygotes that can be easily determined by the visualization of the sparse-fur and wrinkled skin phenotype. Of the 38 progeny produced in this control cross B, 14 were male, and of these, 8 were phenotypically normal and 6 were mutant, as determined by the presence of wrinkled skin and abnormal fur. In the test cross with F1 mouse #213, of the 35 progeny produced in this cross, all eleven of the male progeny were phenotypically normal, clearly showing the genotyping of F1 mouse #213 as a germline transmitted gene corrected homozygous normal female.

As another independent test to determine if the normal gene corrected allele in mouse #16 could be transmitted through the germline, mouse #16 was mated with homozygous (spf-ash/spf-ash) mutant females. In this cross if mouse #16 does not transmit a normal allele, the resultant progeny will either be hemizygous (spf-ash/Y) mutant males or homozygous

(spf-ash/spf-ash) mutant females, both of which are phenotypically mutant. However if the mouse allele is transmitted through the germline, heterozygous (spf-ash/+) females that are phenotypically normal will be produced. When mouse #16 was bred with homozygous (spf-ash/spf-ash) mutant females, two litters were produced that consisted of a total of 5 hemizygous (spf-ash/Y) mutant males, 7 homozygous (spf-ash/spf-ash) mutant females, and 1 phenotypically normal female (mouse #1014). Pictures of representative mice from these crosses are shown in Figure 23. The production of the phenotypically normal female mouse provides direct genetic evidence that mouse #16 contains a normal gene corrected OTC allele that is germline transmissable.

Although the present invention has been described in some detail by way of illustration for purposes of clarity of understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the claims.